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# Gamma Chain Abnormal Human Fetal Hemoglobin Variants

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## INTRODUCTION

In 1968, Schroeder et al. [1] described the chemical heterogeneity of human fetal hemoglobin (Hb F) of the newborn baby; the glycine content of the C-terminal cyanogen bromide peptide, γCB-3, was always less than the expected one residue, while that of alanine was more than the known two residues. Analysis of the glycine content of the γCB-3 peptide, isolated from a few γ chain abnormal variants, showed that some had a ratio of 1.0 to 2.0 and others of 0 to 3.0, while this ratio for normal cord blood Hb F averaged 0.7 to 2.3. These data were interpreted to indicate the existence of two non-allelic γ-globin genes; one was named the $^{G}\gamma$-globin gene and its γ chain product has a glycine residue at position 136, while a second, named the $^{A}\gamma$-globin gene, regulates the synthesis of a γ chain with an alanine residue at that position. The existence of these duplicated γ-globin genes was confirmed in the early 1980s through several studies, including sequence analysis of cloned DNA [2,3], which determined their exact location in the β-globin gene cistron on the short arm of chromosome 11 (additional references in [4]).

The number of γ chain abnormal Hb F variants has been increasing steadily, and at present 69 different abnormalities have been registered [5]; of these 38 are $^{G}\gamma$ variants (glycine at position γ136), 28 are $^{A}\gamma$ variants (alanine at γ136), while for three variants this information is not available. A short review of these interesting proteins is the topic of this communication.

## DETECTION

The great majority of these variants is characterized by an amino acid replacement involving a charged amino acid residue; these abnormalities were detected when cord blood red cell lysates were tested with an electrophoretic procedure such as starch gel or cellulose acetate electrophoresis [6] or isoelectrofocusing (IEF) [7]. Analysis of the parents' hemoglobins (Hbs) with the same procedure is most helpful; they should not have a notable quantity of the variant. About 10 variants have neutral amino acid replacements in their abnormal γ chains, which have been detected by polyacrylamide gel electrophoresis (PAGE) [8,9] or reversed phase high performance liquid chromatography (HPLC) [10,11]. The latter procedure is also ideally suited to separate the normal $^{G}\gamma$ and $^{A}\gamma$ chains.

## CHARACTERIZATION

The amino acid replacement in most variants has been determined through structural analysis of the isolated abnormal Hb F ($F_X$) or the abnormal γ ($\gamma^X$) chain [12,13]. Often, only a small volume of newborn blood is available, complicating the isolation of a sufficient quantity of Hb $F_X$ for analysis. HPLC methodology has greatly facilitated the isolation of the $\gamma^X$ chain and its tryptic peptides [14]. Several Hb $F_X$ variants have been characterized through this approach, which also included the analysis of the γT-15 peptide (positions 133 through 144) allowing the identification of the $\gamma^X$ chain as a variant of the $^{G}\gamma$ or $^{A}\gamma$ chain. A few abnormalities have been found to have two amino acid replacements, one being the common Ile→Thr substitution at position 75 of

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the $^{A}\gamma$ chain ($^{A}\gamma^{T}$ variants). More recently, sequence analysis of amplified DNA which includes the $^{G}\gamma$- or $^{A}\gamma$-globin gene has replaced the protein analysis as a major procedure for the characterization of Hb $F_X$. A few milliliters of cord blood provides a sufficient quantity of genomic DNA, while the same technique can be used for detecting the mutation in one of the parents. References [15–17] give useful technical details. The method is fast and readily identifies the mutation responsible for the specific amino acid replacement as well as the nucleotide changes at codon 136 (GGA for glycine; GCA for alanine), at codon 75 (ATA for isoleucine; ACA for threonine), or at any other location.

## QUANTITATION

Reversed phase HPLC [10,11] is the method of choice for the determination of the percentage of the $\gamma^{X}$ chain (as % of total $\gamma$) provided an adequate separation from the other polypeptide chains can be obtained. Other procedures are DEAE-cellulose chromatography [6], cation exchange HPLC [18], and elution of the appropriate bands after an electrophoretic separation.

## RELATIVE QUANTITIES OF THE $^{G}\gamma$ AND $^{A}\gamma$ VARIANTS IN THE NEWBORN

A review of published and unpublished data has established that acceptable information is available for 34 of the known 38 $^{G}\gamma$ variants and for 24 of the 28 known $^{A}\gamma$ variants. These results are shown in Figure 1 together with the different amino acid replacements. The five $^{A}\gamma$ variants and two $^{G}\gamma$ variants with two amino acid replacements (one being the Ile→Thr exchange at position 75) are also identified. Excluding the five babies with low levels for a specific $^{G}\gamma$ variant and the three babies with high levels for a specific $^{A}\gamma$ variant, the average values at birth are 31% with a range of 23–37% ($^{G}\gamma$) and 13% with a range of 7.5–17% ($^{A}\gamma$); the ratio between these two averages is the same as the 70:30 ratio observed for the $^{G}\gamma$ and $^{A}\gamma$ chains in normal newborn babies.

## $^{G}\gamma$ CHAIN VARIANTS PRESENT IN LOW QUANTITIES

Five such variants have been observed. Two, namely *Hb F-M-Osaka* or $\alpha_2{}^{G}\gamma_2$63(E7)His→Tyr and *Hb F-M-Fort Ripley* or $\alpha_2{}^{G}\gamma_2$92(F8)His→Tyr, are fetal metHbs causing neonatal cyanosis [19–22], probably due to the presence of mixed hybrids ($\alpha_2{}^{G}\gamma^{X}\gamma$) with a low oxygen affinity. These Hb variants are the fetal counterparts of the adult metHbs in which also either the distal or proximal histidine in $\alpha$ or $\beta$ chains are replaced by a tyrosine residue; fortunately, the neonatal cyanosis is relatively

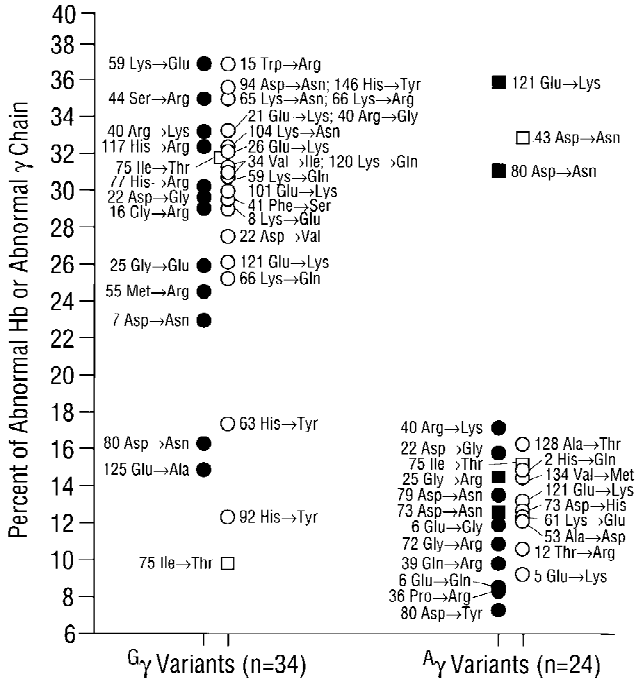


**Fig. 1. The quantities of $^{G}\gamma$ and $^{A}\gamma$ chain variants in heterozygous newborn babies. Open circles: data by reversed phase HPLC; closed circles: data by other procedures (DEAE-cellulose chromatography; electrophoresis; etc.); open and closed squares represent variants with an (additional) amino acid replacement at $^{A}\gamma$75 Ile→Thr ($^{A}\gamma^{T}$ variants).**

mild and disappears when the $\gamma$ chains are replaced by $\beta$ chains. The low levels of these two abnormal $\gamma$ chains are due to the considerable instability that has been observed [22]. *Hb F-Port Royal* or $\alpha_2{}^{G}\gamma_2$125(H3)Glu→Ala was described in 1974 [23], at which time its relatively low level (14–15%) was reported. The variant has been observed at a low frequency in the Black population of the Southeastern United States. We recently sequenced the amplified $^{G}\gamma$- and $^{A}\gamma$-globin genes of several babies with this variant (unpublished data). No mutation was observed in the $^{G}\gamma$-globin genes but two nucleotide changes were present in the $^{A}\gamma$-globin gene: A G**A**G→G**C**G change at codon 125 (responsible for the Glu→Ala replacement) and a G**C**A→G**G**A change at codon 136 (responsible for the Ala→Gly replacement). These observations confirm the assumption that a G**A**G→G**C**G mutation occurred on an $^{A}\gamma$-globin gene in which codon 136 (GCA) introduced a glycine at that position in the abnormal $\gamma$ chain. $\gamma$-Globin gene arrangements of the type -$^{G}\gamma$-$^{G}\gamma$- (instead of -$^{G}\gamma$-$^{A}\gamma$-) have been reported before [24,25] and sequence analyses have confirmed that the G**C**A→G**G**A change is the only mutation that is present in the downstream $^{G}\gamma$ gene [26]. Table I lists data for eight newborns with a Hb F-Port Royal heterozygosity and with different $\gamma$-globin gene arrange-

**TABLE I. Hb Composition Observed in Newborn Babies With a Hb F-Port Royal [$^{G}\gamma$125(H3)Glu→Ala] Heterozygosity**

| | Baby no. | Hb F (%)[a] | Hb $F_x$ (%)[a] | Hb $F_x \cdot 100$ Total Hb F | Hb A (%)[a] | $A_\gamma T$ (%)[b] | $G_\gamma + G_\gamma X$ (%)[b] | $A_\gamma$ (%)[b] |
|---|---|---|---|---|---|---|---|---|
| A[c] | 2,159 | 61.6 | 13.0 | 17.4 | 25.4 | 0 | 81.2 | 18.8 |
| | 903 | 63.8 | 11.7 | 15.5 | 24.5 | 0 | 85.6 | 14.4 |
| | 132 | 79.4 | 10.7 | 11.9 | 9.9 | 0 | 85.9 | 14.1 |
| | 11,162 | 68.9 | 8.3 | 10.8 | 22.8 | 0 | 83.4 | 16.6 |
| | Averages | 68.4 | 10.9 | 13.9 | 20.7 | 0 | 84.0 | 16.0 |
| B[c] | 753 | 70.0 | 13.7 | 16.4 | 16.3 | 18.3 | 81.7 | 0 |
| | 11,289 | 80.4 | 11.5 | 12.5 | 8.1 | 14.7 | 85.3 | 0 |
| | Averages | 75.2 | 12.6 | 14.4 | 12.2 | 16.5 | 83.5 | 0 |
| C[c] | 832 | 63.3 | 12.3 | 16.3 | 24.4 | 0 | 100.0 | 0 |
| | 118 | 70.0 | 13.1 | 15.7 | 16.8 | 0 | 100.0 | 0 |
| | Averages | 66.7 | 12.7 | 16.0 | 20.6 | 0 | 100.0 | 0 |

[a]By cation exchange HPLC [18].
[b]By reversed phase HPLC [10].
[c]A = $^{G}\gamma \cdot {}^{G}\gamma^{X}/{}^{G}\gamma \cdot {}^{A}\gamma$. B = $^{G}\gamma \cdot {}^{G}\gamma^{X}/{}^{G}\gamma \cdot {}^{A}\gamma^{T}$. C = $^{G}\gamma \cdot {}^{G}\gamma^{X}/{}^{G}\gamma \cdot {}^{G}\gamma$.

ments in trans; the level of Hb $F_X$ varied between 10.8 and 17.4% in all eight babies.

*Hb F-Marietta* or $\alpha_2{}^{G}\gamma_2$80(EF4)Asp→Asn has been observed once [27]; it was present in a Caucasian newborn at a level of 16.2% (as % of total Hb F). No further studies have been conducted, but it is tempting to assume that this mutation also occurred on an $^{A}\gamma$-globin gene in which codon 136 was changed from GCA (for alanine) to GGA (for glycine).

*Hb F-Charlotte* or $\alpha_2{}^{G}\gamma_2$75(E19)Ile→Thr occurs in a newborn baby at a level of 10% of total Hb F; the $\gamma^{X}$ chain was discovered by reversed phase HPLC and eluted between the α and β chains [28]. The $^{A}\gamma$ level in this baby was about 28% and the $\gamma^{X}$ chain was considered to be the product of a mutant $^{A}\gamma$ gene with a G**C**A→G**G**A change at codon 136. Interestingly, the same variant has been observed in an Italian baby [29] and a Black baby [30] but at a much higher level of 32.4% (*Hb F-Sassari* in the Italian baby) and 29.2% (*Hb F-Waynesboro* in the Black baby). Both infants were heterozygous for the $^{A}\gamma^{T}$ chain, the $^{A}\gamma$ variant with an Ile→Thr mutation at codon 75, and had low $^{G}\gamma$ values of 37–43%. These data suggest that the $\gamma^{X}$ chain of Hb F-Charlotte is a mutation of the 3′ $^{G}\gamma$-globin gene, and the $\gamma^{X}$ chains of Hb F-Sassari and Hb F-Waynesboro are the products of a mutated 5′ $^{G}\gamma$ gene of the -$^{G}\gamma$-$^{G}\gamma$- arrangement. This was confirmed by sequencing of amplified DNA involving the $^{G}\gamma$-globin gene: codon 75 of the $^{G}\gamma$ gene from the Hb F-Charlotte baby was ATA (for isoleucine) and ACA (for threonine), while this T→C mutation was not present in the $^{G}\gamma$-globin gene of the Hb F-Waynesboro baby. Sequence analyses of the amplified $^{A}\gamma$-globin genes from both babies suggested a limited gene conversion by replacing a segment of the $^{A}\gamma$ gene by that of a $^{G}\gamma$ gene, which made the 3′ end different from that of a normal $^{A}\gamma$ gene. Both hybrid genes have an A**T**A→A**C**A mutation at codon 75 (Ile→Thr), while the hybrid gene in the Hb F-Charlotte baby has an additional GCA (for alanine) to GGA (for glycine) mutation at codon 136. These analyses show that different mechanisms, often based on different mutations and/or gene arrangements, can result in variations of the quantities of the abnormal γ chains [30].

## $^{A}\gamma$ VARIANTS PRESENT IN HIGH QUANTITIES

*Hb F-Sardinia* or $\alpha_2{}^{A}\gamma_2$75(E19)Ile→Thr was discovered in 1975 [31]. Soon it became evident that this amino acid exchange occurred at relatively high frequencies in many populations. The incidence of this anomaly, also known as the $^{A}\gamma^{T}$ chain, has been reviewed twice [15,32]; interestingly, it has been observed in many racial and ethnic groups, excluding the Black population of Africa. For instance, only one of the many haplotypes associated with the presence of the $\beta^{S}$ gene (haplotype 17 or Cameroon) is characterized by the A**T**A→A**C**A mutation at codon 75 of the $^{A}\gamma$-globin gene. Because of its widespread occurrence it seems not surprising that additional mutations are present in some genes located on a chromosome with the $^{A}\gamma^{T}$ change. Five $^{A}\gamma^{T}$ variants have been described; two, namely *Hb F-Xinjiang* or $\alpha_2{}^{A}\gamma^{T}{}_2$25(B7)Gly→Arg [33] and *Hb F-Forest Park* or $\alpha_2{}^{A}\gamma^{T}{}_2$73(E17)Asp→Asn [34], are present in the expected quantities of 12–14%. The other three, however, are present at high levels of 31–36% in the heterozygous newborn; these are *Hb F-Yamaguchi* or $\alpha_2{}^{A}\gamma^{T}{}_2$80(EF4)Asp→Asn [35–37], *Hb F-Fukuyama* or $\alpha_2{}^{A}\gamma^{T}{}_2$43(CD2)Asp→Asn [38], and *Hb F-Siena* or $\alpha_2{}^{A}\gamma^{T}{}_2$121(GH4)Glu→Lys [39]. The high level of Hb F-Yamaguchi is the only one which has been satisfactorily explained; the other two have not been studied in detail.

Hb F-Yamaguchi was discovered in 1981 by Fuyuno et al. [35] in a Japanese newborn and a second observation was made in 1982 [36]. Detailed gene mapping analysis of the latter baby and four of his relatives

showed the deletion of a ~5.1 kb DNA segment (*Bgl* II digestion) in the baby, father, brother, and grandmother [37]. This deletion removes the 3′ part of the $^{G}\gamma$ gene, the intergenic DNA sequence, and the 5′ part of the $^{A}\gamma$ gene. The resulting hybrid gene of this γ-thalassemic type of condition produces at birth an $^{A}\gamma$ chain at a level of $^{G}\gamma$, probably because of a $^{G}\gamma$ promoter sequence, thus explaining the high level of Hb F-Yamaguchi. This form of γ-thal without the presence of the Hb F-Yamaguchi mutation has been observed before [40,41]; it is characterized by a decreased level of total Hb F, which is about 50% in the two homozygotes that have been discovered. Interestingly, the hybrid gene (often indicated as -$^{G}\gamma^{A}\gamma$-) of the $^{G}\gamma \cdot {}^{A}\gamma$/-$^{G}\gamma^{A}\gamma$- arrangement does not always carry the $^{A}\gamma^{T}$ mutation (i.e., A**T**A→A**C**A at codon 75) [41]. Perhaps two independent crossovers, one involving an $^{A}\gamma$-globin gene with the $^{A}\gamma^{T}$ mutation and the other without this change, resulted in two different types of γ-thal which cannot be distinguished by the gene mapping procedure that was used in the reported studies. Observations at the protein level in the baby with the Hb F-Fukuyama heterozygosity [38] made Hidaka et al. suggest that the higher production of this $^{A}\gamma^{T}$ chain with the Asp→Asn replacement at position 43 could have arisen as a result of the presence of a γ-thal similar to that observed for the Hb F-Yamaguchi heterozygote.

## CONCLUSIONS

The -$^{G}\gamma$-$^{A}\gamma$- arrangement is one of the three sets of globin gene duplications; the others are the -α-α- and the -δ-β- arrangements. The products of the α-globin genes are the same, those of the γ-globin genes differ in one amino acid residue, while the δ-β-globin genes regulate the synthesis of two polypeptides, which differ in 10 positions [reviewed in 42]. Furthermore, the rates of synthesis differ particularly for the δ-β-globin gene system where the production of the δ chain is impaired because of a defective δ promoter sequence [reviewed in 43]. At birth, the $^{G}\gamma$-globin gene is more active than the $^{A}\gamma$-globin gene, but this is gradually changing with time, unless a C→T mutation at position −158 or a G→A mutation at position −161 is present in the $^{G}\gamma$-globin gene promoter [44–46]. Abnormal Hbs with variations in either one of these three arrangements are well known; but those in the $^{G}\gamma$- and $^{A}\gamma$-globin genes are the least well studied, primarily because methodology used in past experiments was often not satisfactory for detailed analyses. With the advance of DNA methodology this is rapidly changing. Besides simple point mutations, a gene conversion, a deletion, and a hybrid gene have been identified. Application of these techniques to the γ-globin system of newborn babies with unusual variants and of their parents will likely result in the detection of additional abnormalities, which cannot be characterized by protein analysis alone.

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